ENZYMATIC OXIDATION OF ALPHA-1-PROTEINASE INHIBITOR IN ABNORMAL TISSUE TURNOVER

This review paper describes present knowledge with regard to the interaction of alpha-1-proteinase inhibitor (α_1-PI) and myeloperoxidase *in vitro*, using both purified enzyme and polymorphonuclear leukocytes as study souces. In the first place, α_1-PI was found to contain a methionyl residue at its reactive center which was sensitive to oxidation by either N-chlorosuccinimide or by enzymatic oxidation. Chemical oxidation revealed that two of the eight methionyl residues present in the molecule were oxidized, one of which was at the reactive center. Subsequently, it was found that the enzymatic oxidation by myeloperoxidase from neutrophil granules, in the presence of hydrogen peroxide and halide ion, could also be easily attained. Since myeloperoxidase is present in the same granules which contain proteolytic enzymes, it would be expected that both types of enzymes would be released simultaneously during either phagocytosis or cell turnover. It is worth noting that the myeloperoxidase system does not oxidize or inactivate most other proteinase inhibitors or proteinases. In the second place, it was shown recently that when α_1-PI was exposed to viable neutrophils in the presence of chloride ion and phorbol myristyl acetate, the abiltiy of α_1-PI to bind elastase was diminished. However, when myeloperoxidase-deficient cells or those from patients with chronic granulomatous disease were used, there was no effect on α_1-PI activity, indicating the involvement of myeloperoxidase and hydrogen peroxide in α_1-PI inactivation. More recently, oxidized α_1-PI has been isolated from rheumatoid synovial fluid of arthritic patients and also from lung lavage fluids of smokers. Approximately four methionyl residues were found to be oxidized in either case, compared to two oxidized residues when native α_1-PI was treated with either chemical or enzymatically produced oxidants. The indication is, therefore, that oxidative inactivation of α_1-PI does occur *in vivo*. Other studies discussed here deal with the importance of oxidation to emphysema development, the actions of the myeloperoxidase system, and the possible role of macrophages in the oxidation of α_1-PI.

Matheson, N. R., Janoff, A. and *Travis, J.*

Molecular and Cellular Biochemistry 45:65-71, 1982.

From the Department of Biochemistry, University of Georgia, Athens, and the Department of Pathology, State University of New York at Stony Brook, Stony Brook.

RAPID CONVERSION OF ANGIOTENSIN I TO ANGIOTENSIN II BY NEUTROPHIL AND MAST CELL PROTEINASES

Angiotensin II is a peptide that has potent vasoconstrictor and aldosterone secretion activities and is derived from the larger parent protein angiotensinogen, which circulates in the plasma. On the other hand, angiotensin I is a peptide that has no significant biological activity and requires further conversion to angiotensin II. In the studies reported here, it is shown that human neutrophil cathepsin G and human skin mast cell chymase rapidly convert angiotensin I to angiotensin II with only minor cleavage elsewhere in the molecule. The rate of cleavage is consistent with a potential role for either or both of these enzymes in an alternate pathway for angiotensin II

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synthesis. Since neither enzyme is inhibited by captopril, an angiotensin converting enzyme inactivator, it is possible that leukocyte and mast cell enzymes may play a significant role in the development of abnormally high local concentrations of angiotensin II, associated with various inflammatory processes.

Reilly, C. F., Tewksbury, D. A., Schechter, N. M., and *Travis, J.*

The Journal of Biological Chemistry 257(15):8619-8622, 1982.

Other support: National Institutes of Health.

From the Department of Biochemistry, University of Georgia, Athens, the Marshfield Foundation for Medical Research, Marshfield, WI, and the Department of Medicine, Duke University School of Medicine, Durham, NC.

THE EFFECT OF α_2-MACROGLOBULIN ON THE INTERACTION OF α_1-PROTEINASE INHIBITOR WITH PORCINE TRYPSIN

The dissociation of α_1-proteinase inhibitor:porcine trypsin complex is rapid and has permitted the experiments reported here. In this study, the rate of dissociation of the α_1-proteinase inhibitor:porcine trypsin complex by itself was compared with the rate of dissociation of the same complex in the presence of α_2-macroglobulin. In the presence of the latter inhibitor the dissociation was more rapid, and active α_1-proteinase inhibitor could be recovered in the mixture. However, no active inhibitor could be detected after dissociation in the absence of α_2-macroglobulin. This recovery of active α_1-proteinase inhibitor from complexes with porcine trypsin is the first demonstration of a thermodynamic equilibrium between this inhibitor and proteinase. Consequently, the transfer of trypsin from complexes with α_1-proteinase inhibitor to α_2-macroglobulin may be explained as a passive phenomenon which does not require a physical collision between α_2-macroglobulin and the α_1-proteinase inhibitor:porcine trypsin complex. The dissociation of the complex occurs more rapidly in the presence of α_2-macroglobulin because this inhibitor complexes trypsin leaving the α_1-proteinase inhibitor:porcine trypsin complex by both the irreversible breakdown step and by reversible dissociation of the complex.

Beatty, K., *Travis, J.* and Beith, J.

Biochimica et Biophysica Acta 704:221-226, 1982.

Other support: National Institutes of Health.

From the Department of Biochemistry, University of Georgia, Athens, and the Faculté de Pharmacie, Laboratoire d'Enzymologie, Université Louis Pasteur, Strasbourg, France.

ENZYMATIC REDUCTION OF OXIDIZED α-1-PROTEINASE INHIBITOR RESTORES BIOLOGICAL ACTIVITY

Oxidation easily inactivates α_1-proteinase inhibitor (α_1-PI), the major serum inhibitor of proteolytic activity. The noted inactivation seems to be due to the oxida-

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tion of an essential methionine residue(s) in α_1-PI that is required for the inhibition of elastase activity. When methionine residue(s) in α_1-PI are oxidized, they result in Met(0). In theory, the presence of an enzyme in cells that can reduce Met(0) residues in proteins to methionine provides a mechanism for restoring biological activity to a protein that has been inactivated because of oxidation of an essential methionine residue. It has been shown elsewhere that extracts of *Escherichia coli* contain an enzyme that can reduce Met(0) residues in protein to methionine. This study shows that oxidized, functionally inactive canine α_1-PI does indeed regain its biological inhibitory activity after reduction with a partially purified preparation of *E. coli* Met(0) peptide reductase.

Abrams, W. R. *Weinbaum, G.*, Weissbach, L., Weissbach, H., and Brot, N.

Proceedings of the National Academy of Sciences of the United States of America 78(12):7483-7486, 1981.

Other support: National Heart, Lung, and Blood Institute.

From the Department of Medicine, Albert Einstein Medical Center, Philadelphia; Department of Biochemistry and Molecular Biology, University of Florida, Gainesville; and Roche Institute of Molecular Biology, Nutley, NJ.

NEUTROPHIL DEGRANULATION IN CADMIUM CHLORIDE-INDUCED ACUTE LUNG INFLAMMATION

Cadmium chloride ($CdC1_2$), a toxic chemical that has been reported to cause centrilobular or scar emphysema, was used in this study to induce neutrophil migration into the alveolar spaces as a model of lung inflammation. Results showed that lobar intrabronchial instillation of $CdC1_2$ (200 μg/ml) in saline causes a reproducible acute pulmonary inflammation in dogs. The influx of inflammatory neutrophils from the circulation into the alveolar spaces reaches a maximum approximately 16 hours after the $CdC1_2$ treatment in the treated lobe, while the contralateral lung appears normal. Morphometric quantitation of peroxidase-positive (azurophilic) granules in the inflammatory neutrophils shows a 74% loss of these granules, with little or no loss of the peroxidase-negative (specific) granules. These data are in good agreement with the measured loss of intracellular elastase, an enzyme known to be localized in the azurophilic granules. The results suggest that degranulation of azurophilic granules may occur selectively during this chemically-induced acute inflammation.

Yamada, H., Damiano, V. V., Tsang, A-L., Meranze, D. R., Glasgow, J., Abrams, W. R., and *Weinbaum, G.*

American Journal of Pathology 109:145-156, 1982.

Other support: National Heart, Lung, and Blood Institute.

From the Research Division, Department of Medicine, Albert Einstein Medical Center, Philadelphia, and the Franklin Research Center, Philadelphia.

PARASYMPATHETIC INNERVATION OF THE CERVICAL TRACHEALIS MUSCLE IN LIVING DOGS

Use of the trachea to study airway function in living dogs permits examination of a prototypic central airway under relatively convenient circumstances using established

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technology. In the study presented here, the pathways by which parasympathetic fibers were carried to the cervical trachaelis muscle were characterized in 34 anesthetized dogs. Nerves were stimulated electrically, and tracheal tension was monitored in segments of the posterior membrane *in vivo*. Stimulation of superior laryngeal nerves contracted 34 of 34 cranial cervical segments and two of four caudal cervical segments. Recurrent laryngeal nerves contributed to innervation of 34 of 34 cranial, as well as four of four caudal, segments. Stimulation of paracurrent nerves contracted 11 of 34 cranial and four of four caudal segments. Mechanical effects of esophageal contraction, induced by stimulating pararecurrent nerves, did not alter tension in tracheal segments. Tracheal contractions induced by stimulation of all three pathways simultaneously were significantly less than the sum of contractions produced by stimulating each set individually; this was probably due to anastomoses between terminal neurons, overlap in their anatomic distribution, or intercellular nexuses in trachealitis muscle. It seems, therefore, that parasympathetic innervation of the canine trachea is by three different neuroanatomic pathways.

Brown, J. K., Shields, R. and *Gold, W. M.*

Journal of Applied Physiology: Respirat, Environ. Exercise Physiol. 53(3):617-625, 1982.

Other support: National Heart, Lung and Blood Institute.

From the Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco.

EFFECT OF SMOKING A CIGARETTE ON THE DENSITY DEPENDENCE OF MAXIMAL EXPIRATORY FLOW

Earlier studies of these investigators have shown that tobacco smoke causes an increase in airways resistance and a drop in expiratory flow at 50% of the vital capacity (FEF_{50}). In the present attempt to gain further insight into the nature and site of this bronchoconstrictive effect, maximal expiratory flow was measured in 12 healthy volunteers while they were breathing air and a low-density gas mixture (helium-oxygen) before and after smoking a cigarette. Results showed that prior to smoking the forced vital capacity (FVC) measured while breathing air was not significantly different from that obtained while breathing the helium-oxygen mixture. Smoking did not cause any changes in FVC. However, after smoking, the FEF_{50} measured while breathing air decreased significantly, while smoking did not cause any changes in the FEF_{50} obtained after breathing the low-density gas mixture. $\Delta\dot{V}max_{50}$ increased from 47.1± 11.4% before smoking to 57.0 ± 13.3% after smoking. There were no changes in flow at 75% FVC and volume of isoflow. These observations are discussed in light of the equal pressure point (EPP) analysis and wave speed theory of flow limitation. It was concluded that after smoking, flow becomes more density dependent because there is constriction of a flow-limiting segment downstream from the EPP, located in lobar and segmental bronchi. No acute effect of tobacco smoke on the small airways could be demonstrated.

Taveira Da Silva, A. M. and *Hamosh, P.*

Respiration 43:258-262, 1982.

From the Departments of Medicine, Physiology and Biophysics, Georgetown University Schools of Medicine and Dentistry, Washington, DC.

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RESPIRATORY AND CARDIOVASCULAR EFFECTS OF INTRAVENTRICULAR CHOLECYSTOKININ

In this attempt to assess the role of cholecystokinin- and gastrin-like peptides in the central, regulatory control of respiratory and cardiovascular functions, cholecystokinin in doses of 1-300 ng was administered into the lateral brain ventricle of chloralose-anesthetized cats while tracheal airflow, arterial blood pressure, and heart rate were monitored. Results showed that the most striking effect was an increase in respiratory activity. This was observed with a dose as low as 1 ng and peaked with a dose of 100 ng. Respiratory stimulation was indicated by an increase in respiratory minute volume, an increase that was due to an increase in tidal volume as no significant effect was noted on respiratory rate, inspiratory time, expiratory time, and total respiratory cycle duration. On the other hand, when 300-1000 ng of cholecystokinin were administered intravenously, no respiratory stimulant effect was observed. These results indicate that cholecystokinin acts in the brain to stimulate respiration.

Pagani, F. D., Taveira Da Silva, A. M., *Hamosh, P.*, Garvey, T. Q., III and Gillis, R. A.

European Journal of Pharmacology 78:129-132, 1982.

Other support: American Heart Association.

From the Departments of Pharmacology, Physiology and Medicine, Georgetown University Schools of Medicine and Dentistry, Washington, DC.

CIGARETTE SMOKE CONTAINS ANTICOAGULANTS AGAINST FIBRIN AGGREGATION AND FACTOR XIIIa IN PLASMA

In this *in vitro* study, the effect of cigarette smoke on fibrin aggregation was investigated by the use of water-soluble, gas-phase components of smoke, obtained by bubbling the smoke produced from one cigarette through three ml of buffer or distilled water. This extract was incorporated in varying dilutions in the buffer to which fibrin monomer solution was added in order to initiate fibrin aggregation. Results showed a dose-dependent delay in fibrin aggregation. Increasing the amount of smoke extract resulted in decreased absorbance of the clot and delayed onset of fibrin aggregation. The fibrin aggregation inhibitor was also examined by use of two differing fibrin preparations with α chains lacking COOH-terminal segments. From the sum of these studies, it was seen that cigarette smoke contains two distinct coagulation inhibitors: one which prolongs the clotting times of plasma by inducing delayed fibrin aggregation and requiring the COOH-terminal region of fibrin α chains to exert its effect; the other inactivates XIIIa, thus preventing the cross-linking of fibrin polymers. These anticoagulant properties of smoke are demonstrable in plasma, where they may play a role in the physiology of smoking.

Galanakis, D. K., Laurent, P., *Janoff, A.*, and Chung, S. I.

Science 217:642-645, 1982.

Other support: U. S. Public Health Service.

From the Health Science Center, State University of New York at Stony Brook, Stony Brook, and the National Institute of Dental Research, Bethesda, MD.

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PLATELETS INCREASE NEUTROPHIL ADHERENCE *IN VITRO* TO NYLON FIBER

Interaction of neutrophils with platelets is a common phenomenon that may be important in the pathogenesis of various diseases. Although platelets are known to have a strong affinity for foreign surfaces, their effect on the measured adherence of neutrophils in the commonly used nylon fiber systems had not been determined. Therefore, in the study reported here, the effect of platelets on the adherence of neutrophils to nylon fiber was assessed in whole blood samples and purified neutrophil suspensions in the presence or absence of plasma. Measurements showed that in whole blood samples, increasing numbers of platelets were associated with increasing adherence of neutrophils. Addition of platelets in plasma to purified neutrophil suspensions increased ($p<0.05$) neutrophil adherence from 76.2% ± 1.4 to 88.0% ± 2.0. Similarly, addition of washed platelets without plasma also increased ($p<0.05$) neutrophil adherence from 67.9% ± 5.8 (without added platelets) to 94.2% ± 1.6 (with 300,000 platelets/mm³ added). In contrast, no augmentation of neutrophil adherence occurred if platelets had their aggregation response suppressed by pretreating platelet donors with aspirin. Scanning electron microscopy supported these findings by showing platelets in close association with neutrophils adhering to nylon fiber. These findings emphasize the importance of platelet numbers and reactivity on the adherence of neutrophils.

Rasp, F. L., Clawson, C. C. and *Repine, J. E.*

The Journal of Laboratory and Clinical Medicine 97(6):812-819, 1981.

Other support: American Lung Association, Minnesota Medical Foundation, National Institutes of Health, American Heart Association, Kroc Foundation, and the Graduate School of the University of Minnesota.

From the Departments of Medicine and Pediatrics, University of Minnesota Health Sciences Center, Minneapolis, and the Webb-Waring Lung Institute and Department of Internal Medicine (Pulmonary), University of Colorado Health Sciences Center, Denver.

A NOVEL MECHANISM FOR PULMONARY OXYGEN TOXICITY: PHAGOCYTE MEDIATED LUNG INJURY

In initial studies, a significant increase in the number of polymorphonuclear leukocytes (PMN) in the alveolar lavages of rats exposed to hyperoxia for three days was noted. This observation led to two hypotheses. The first hypothesis, that hyperoxia causes injury to alveolar macrophages (AM), inducing their release of chemotactic factors which recruit PMNs to the lung, was supported by evidence that: (1) hyperoxia damages AM *in vivo* and *in vitro*, and (2) chemotactic factors from alveolar lavages of hyperoxia-exposed animals are biochemically similar to those released by AM exposed to hyperoxia in cell culture. The second hypothesis, that PMNs play an important role in pulmonary oxygen toxicity, was supported by the finding that the degree of endothelial damage was highly correlated with the numbers of PMNs recovered in alveolar lavages in both neutrophil-sufficient and neutropenic rabbits. However, two major

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unknowns still remain. First, how does hyperoxia damage AM? Second, how do PMNs damage endothelium? At this time, a reasonable working hypothesis is that the damage in each case is due to the formation of oxygen radicals, particularly the highly reactive hydroxyl radicals. Improved understanding of the role of AM, PMN and hydroxyl radical in lung damage due to hyperoxia will be important in elucidating basic mechanisms involved in the pathogenesis of pulmonary oxygen toxicity, as well as in other types of environmental lung injury.

Fox, R. B., Shasby, D. M., Harada, R. N., and *Repine, J. E.*

CHEST 80S:3S-4S, 1981.

Other support: American Heart Association, National Institutes of Health, and the Maytag-Crawford Trust Fund.

From the Experimental Medicine Division, Webb-Waring Lung Institute, University of Colorado Health Sciences Center, Denver.

POTENTIAL MECHANISMS OF LUNG INJURY FROM HYDROXYL RADICAL

Earlier biochemical studies have indicated that inhaled environmental toxins may, in one way or another, contribute to lung damage. For example, there is evidence that polymorphonuclear leukocytes (PMNs) are efficient at making highly reactive O_2^- intermediates and since factors released from alveolar macrophages (AM) exposed to hyperoxia can stimulate release of O_2^- intermediates from PMN, it appears likely that O_2^- intermediates are involved in this injury. More recent work has focused on the killing of bacteria by PMN as a model of PMN-induced lung damage. In one of these studies, increasing concentrations of DMSO or thiourea progressively and significantly decreased killing of *Staphylococcus aureus*, 502A, by normal PMN but did not further decrease the abnormal bactericidal activity of CGD PMN which fail to produce •OH. For studies with Fe^{++} it was hypothesized that *S. aureus* was providing a majority of the substances(s), such as Fe^{++}, which then reacted with H_2O_2 to generate •OH and CH_4 from DMSO. The results of these studies indicate that hydroxyl radical (•OH) plays an important role in the killing of *S. aureus* by human PMN. Other studies suggested that •OH might also be formed and participate in the killing of *S. aureus* in another way. Elucidation of this latter mechanism was an outgrowth of studies done to determine the bactericidal activity of chemical system, Fe^{++} and H_2O_2, which generates •OH. In these studies it appears that H_2O_2 reacts with Fe^{++} in the bacteria to produce •OH by a Fenton type reaction. Hydroxyl radical then injures *S. aureus* by reacting with key organic molecules. It was also noted here that DMSO prevents injury to *S. aureus* by scavenging and detoxifying •OH.

Repine, J. E. et al.

CHEST 80S:45S-48S, 1981.

Other support: American Heart Association, National Institutes of Health, and the Maytag-Crawford Trust Fund.

From the Webb-Waring Lung Institute and the Division of Pulmonary Medicine, University of Colorado Health Sciences Center, Denver.

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HYDROGEN PEROXIDE KILLS *STAPHYLOCOCCUS AUREUS* BY REACTING WITH STAPHYLOCOCCAL IRON TO FORM HYDROXYL RADICAL

In the investigation reported here, an attempt was made to elucidate the mechanism underlying the biochemical role of iron in bacterial host-defense interactions. Results showed that two different lines of investigation supported the premise that killing of *Staphylococcus aureus*, 502A, by hydrogen peroxide involves formation of the more toxic hydroxyl radical (•OH) through the iron-dependent Fenton reaction. First, growing *S. aureus* overnight in broth media with increasing concentrations of iron increased their content of iron and dramatically enhanced their subsequent susceptibility to killing by H_2O_2. Second, in direct relation to their effectiveness as •OH scavengers, thiourea, dimethyl thiourea, sodium benzoate, and dimethyl sulfoxide inhibited H_2O_2-mediated killing of *S. aureus*. Therefore, it seems from this work that, while iron is an essential growth nutrient, it may also provide an "Achilles heel" for *S. aureus*, indicating that the reported mechanism may be a new way in which iron is beneficial to the host.

Repine, J. E. Fox, R. B. and Berger, E. M.

The Journal of Biological Chemistry 256(14):7094-7096, 1981.

Other support: National Institutes of Health, American Heart Association, and the Kroc Foundation.

From the Webb-Waring Lung Institute and the Pulmonary Divisions of the Departments of Medicine and Pediatrics, University of Colorado Health Sciences Center, Denver.

SERUM FROM PATIENTS WITH INVASIVE FUNGAL INFECTIONS INHIBITS THE ADHERENCE OF POLYMORPHONUCLEAR LEUKOCYTES AND ALVEOLAR MACROPHAGES

Although considerable attention has been directed recently toward determining the effect of bacterial infection on neutrophil adherence, little attention has been paid so far to the influence of fungal infection on phagocyte adherence. In the investigation reported here, however, the adherence of various combinations of polymorphonuclear leukocytes (PMN) or alveolar macrophages (AM) and serum from patients with fungal infections or from control subjects was evaluated *in vitro* using the standard nylon fiber pipette technique. Results showed that the intrinsic adherence of AM and PMN from patients with a wide variety of untreated fungal infections was normal, but that these patients had a serum inhibitor that could reversibly decrease the adherence of PMN and AM. Specifically, studies using various combinations of PMN and serums from patients with blastomycosis suggested that the adherence defect was due to a serum disorder rather that an intrinsic cellular abnormality. While preincubation in serum from patients with blastomycosis decreased the adherence of control PMN, preincubation in control serum corrected the decreased adherence of PMN from patients with blastomycosis. On the basis of these and other related studies, it was concluded that the intrinsic adherence of PMN and AM from patients with untreated fungal infections is normal, but that these patients have an extrinsic heat-labile serum factor that can decrease the adherence of PMN and AM.

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Rasp, F. L., Sarosi, G. A. and *Repine, J. E.*

American Review of Respiratory Disease 123:636-639, 1981.

Other support: Minnesota Medical Foundation, Minnesota and American Lung Associations, National Institutes of Health, American Heart Association, and the Kroc Foundation.

From the Departments of Medicine of the University of Minnesota Health Sciences Center and the Minneapolis Veterans Administration Medical Center, and the Webb-Waring Lung Institute and the Department of Medicine of the University of Colorado Health Sciences Center, Denver.

ANGIOTENSIN CONVERTING ENZYME CONCENTRATIONS IN THE LUNG LAVAGE OF NORMAL RABBITS AND RABBITS TREATED WITH NITROGEN MUSTARD EXPOSED TO HYPEROXIA

Granulocytes have been implicated recently in the development of acute hyperoxic lung injury, an edematous lung mishap that is characterized grossly by massive edema and histologically by endothelial injury with perivascular inflammation. In the present study, specifically, increased concentrations of angiotensin converting enzyme (ACE) were found in lung lavages from rabbits exposed for 72 hours to hyperoxia and the concentrations of ACE were correlated with ratios of extravascular lung water to body weight and albumin concentrations in lung lavages. In parallel studies, rabbits treated with nitrogen mustard in which granulocytopenia was maintained throughout the 72-hour hyperoxic exposure period had less evidence of edematous lung injury and lower concentrations of ACE in their lung lavages than similarly treated rabbits in which granulocytopenia was not maintained. These results suggested that granulocytes contribute to acute edematous lung injury from hyperoxia and that ACE concentrations in lung lavages reflect this process.

Shasby, D. M., Shasby, S. S., Bowman, C. M., Fox, R. B., Harada, R. M., Tate, R. M., and *Repine, J. E.*

American Review of Respiratory Disease 124:202-203, 1981.

Other support: National Heart, Lung and Blood Institute, American Heart Association, National Institutes of Health, and the Kroc Foundation.

From the Webb-Waring Lung Institute, Department of Medicine (Pulmonary Sciences), University of Colorado Health Sciences Center, Denver.

NEUTROPHILS AND LUNG EDEMA: STATE OF THE ART

This brief review focuses on the emerging role of neutrophils and their products in the development of adult respiratory distress syndrome (ARDS), the most common presenting form of edematous lung injury. For some time, the association of ARDS with multiple inciting events has led to the presumption that, under certain circumstances, many factors might be involved in the pathogenesis of ARDS. While not firmly established, a loss of alveolar-capillary membrane integrity which results in lung edema appears to be a pathologic change common to all cases of ARDS, thus

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making it possible for cellular or humoral components to contribute to endothelial injury. A number of recent observations suggest that neutrophils contribute to the development of edematous lung injury. First, increased numbers of neutrophils are commonly found in lung lavages of patients with ARDS and early in their illness. Second, neutrophil depletion protects animals from experimental edematous lung injury, and third, neutrophils have been shown to have potent mechanisms for causing tissue injury, altering vascular permeability and perturbing hemodynamics. Overall, considerable evidence is now available to suggest that neutrophils participate in the development of acute edematous lung injury such as that seen in patients with ARDS. The mechanisms by which neutrophils might mediate these effects appear to be many, varied and still ill-defined at this time.

Repine, J. E., Bowman, C. M., and Tate, R. M.

CHEST 81S:47S-50S, 1982.

Other support: National Institutes of Health, American Heart Association and the Kroc Foundation.

From the Webb-Waring Lung Institute, University of Colorado Health Sciences Center, Denver.

ALVEOLAR MACROPHAGE SECRETIONS: INITIATORS OF INFLAMMATION IN PULMONARY OXYGEN TOXICITY?

In the paper presented here, attempts were made to elucidate mechanisms responsible for neutrophil influx into the lungs and to understand more clearly the contribution of neutrophils to the undefined pathogenesis of pulmonary oxygen toxicity. For this study, alveolar macrophages (AM), following lavage from lungs of infection-free New Zealand White rabbits, were exposed in culture to normoxia (15% O_2) or hyperoxia (95% O_2) for periods up to 72 hrs. Supernatants from AM cultures were evaluated for their chemotactic, adherence stimulating, and superoxide radical stimulating activities for neutrophils. Results showed that hyperoxia damages and stimulates alveolar macrophages to release factors which affect neutrophil recruitment, adherence and activation. Preliminary characterization of these factors suggests that the factors have separate identities. Factors derived from AM exposed to hyperoxia differ in molecular weight, heat stability, and time of maximal activity. The stimulus for macrophage release of these factors may involve damage to AM by hyperoxia. These observations support the possibility that AM and the proposed mechanism of neutrophil recruitment and activation may be important in the pathogenesis of oxygen toxicity and other forms of acute lung injury.

Harada, R. N., Bowman, C. M., Fox, R. B., and *Repine, J. E.*

CHEST 81S:52S-54S, 1982.

Other support: American Lung Association of Colorado, American Heart Association, National Institutes of Health, the Kroc Foundation, Hill Foundation, Swan Foundation, and Kleberg Foundation.

From the Webb-Waring Lung Institute, Pulmonary Divisions, University of Colorado Health Sciences Center, Denver.

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OXYGEN RADICAL-INDUCED PULMONARY EDEMA: A MECHANISM FOR THE PRODUCTION OF NONCARDIOGENIC PULMONARY EDEMA BY NEUTROPHILS

Since stimulated neutrophils make O_2 radicals and since O_2 radicals have been shown to damage a number of biological tissues, it seemed possible that release of O_2 radicals from stimulated neutrophils might perturb the alveolar-capillary membrane and lead to protein-rich edema formation. To test this hypothesis, isolated perfused lungs from New Zealand White rabbits were monitored and, after a stable baseline period, purine and xanthine oxidase were introduced into the perfusate with or without prior introduction of O_2 radical scavengers. Results of this investigation showed that the chemical generation of O_2 radicals by intravascular injection of purine and xanthine oxidase resulted in acute protein-rich edema formation in the isolated lungs. This edematous process was markedly inhibited by the prior individual injection of several O_2 radical scavengers, including catalase, dimethyl sulfoxide, and dimethylthiourea. These findings provide direct evidence to suggest that O_2 radicals are capable of perturbing the air-blood barrier and causing a protein-rich edema. They also suggest that the intra-vascular release of O_2 radicals from stimulated neutrophils might be capable of producing a similar acute edematous lung injury in certain clinical settings.

Tate, R. M., Shasby, D. M., VanBenthuysen, K. M., McMurthy, I. F., and *Repine, J. E.*

CHEST 81S:57S-59S, 1982.

Other support: American Lung Association of Colorado, American Heart Association, National Institutes of Health, the Kroc Foundation, Hill Foundation, Swan Foundation, and the Kleberg Foundation.

From the Webb-Waring Lung Institute, Pulmonary Division, Cardiovascular Pulmonary Research Laboratory and the Departments of Medicine and Pediatrics, University of Colorado Health Sciences Center, Denver.

REDUCTION OF THE EDEMA OF ACUTE HYPEROXIC LUNG INJURY BY GRANULOCYTE DEPLETION

The relationship between granulocytes in the lung and the edema of acute hyperoxic lung injury was examined in the study reported here. Results showed that although increased numbers of granulocytes are found in lungs acutely injured by hyperoxia, their contribution to lung injury remains unknown. It was found that circulating granulocytes increased markedly in rabbits exposed to hyperoxia for 72 hrs. and that the number of granulocytes in lung lavages also increased and were correlated with the degree of edematous lung injury. Furthermore, when rabbits were treated with nitrogen mustard (1.75 mg/kg) and developed sustained granulocytopenia, exposure to hypertoxia for 72 hrs. resulted in fewer granulocytes in lung lavages and less edematous lung injury. In contrast, when rabbits were similarly treated with nitrogen mustard but did not maintain sustained granulocytopenia throughout the exposure to hyperoxia, increased numbers of granulocytes were found in lung lavages and the degree of

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edematous lung injury increased to levels not different from those observed in oxygen-exposed rabbits that had not been treated with nitrogen mustard. These findings suggest that granulocytes may contribute to production of edema in acute oxygen toxicity.

Shasby, D. M., Fox, R. B., Harada, R. N. and *Repine, J. E.*

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 52 (5): 1237-1244, 1982.

Other support: National Heart, Lung and Blood Institute, American Heart Association, and the Kroc Foundation.

From the Webb-Waring Lung Institute, Department of Internal Medicine (Pulmonary Sciences Division), University of Colorado Health Sciences Center, Denver.

GRANULOCYTES MEDIATE ACUTE EDEMATOUS LUNG INJURY IN RABBITS AND IN ISOLATED RABBIT LUNG PERFUSED WITH PHORBOL MYRISTATE ACETATE: ROLE OF OXYGEN RADICALS

Many forms of acute noncardiogenic lung edema are associated with an accumulation of inflammatory cells in the alveoli and microvessels of the lung, and there have been many suggestions that the activated granulocytes play an important role in this process. In the study reported here explicitly, it was seen that intravenously injected phorbol myristate acetate (PMA) caused a protein-rich edema in lungs of control rabbits but not in granulocytopenic rabbits pretreated with nitrogen mustard. Specifically, control rabbits treated with PMA had higher lung weight to body weight ratios and lung lavage albumin concentrations than granulocytopenic rabbits pretreated with nitrogen mustard and then given PMA. To further clarify the role of granulocytes in the production of edema, additional experiments were conducted in an isolated perfused rabbit lung. Addition of purified granulocytes and PMA to the balanced salt perfusate caused lung edema, whereas neither granulocytes nor PMA alone caused edema. Also, in order to determine the contribution of oxygen radicals to the pathogenesis of the edema, chronic granulomatous disease granulocytes, which are deficient in oxygen radical production, were added to the isolated lung perfusate. Chronic granulomatous disease granulocytes and PMA did not cause edema in isolated lungs, whereas granulocytes from normal human subjects and PMA did. These data suggest that oxygen radicals released from stimulated granulocytes contribute to the pathogenesis of acute edematous lung injury.

Shasby, D. M., VanBenthuysen, K. M., Tate, R. M., Shasby, S. S., McMurtry, I., and *Repine, J. E.*

American Review of Respiratory Disease 125:443-447, 1982.

Other support: American Heart Association, National Institutes of Health, and the Kroc Foundation.

From the Webb-Waring Lung Institute and the Cardiovascular Pulmonary Research Laboratory of the University of Colorado Health Sciences Center, Denver, and the Pulmonary Division of the University of Virginia School of Medicine, Charlottesville.

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CYTOCHALASIN B AND THE STRUCTURE OF ACTIN GELS II. FURTHER EVIDENCE FOR THE SPLITTING OF F-ACTIN BY CYTOCHALASIN B

Cytochalasin B, a fungal metabolite that can alter cell shapes and inhibit a wide variety of cellular movements under certain conditions, has been shown in the past to be able to reduce the network structure of actin filaments. In this communication, additional evidence is presented that cytochalasin B shortens actin filaments and that this shortening takes place without net depolymerization. Overall, the work done here showed that cytochalasin B decreased the flow birefringence and $S_{20,w}$ and increased the extinction angle of actin filaments in salt solutions favoring polymerization of the protein. These changes occurred without a detectable increase in the equilibrium actin monomer concentration determined by a radioassay. These results complement earlier observations indicating that cytochalasin B shortens actin filaments without net depolymerization. Analyzed in terms of Flory's classical network theory, this shortening accounts for the marked effect of cytochalasin B in dissolving the gel structure of F-actin crosslinked by actin-binding protein concentration for incipient gelation. Cytochalasin B decreased the annealing rate of low concentrations of actin filament fragments prepared by sonic disruption. This result is consistent with the idea that cytochalasin B binds to the ends of actin filaments, and may explain how cytochalasin B causes filament shortening.

Maruyama, K., Hartwig, J. H. and *Stossel, T. P.*

Biochimica et Biophysica Acta 626:494-500, 1980.

Other support: U. S. Public Health Service.

From the Department of Muscle Research, Boston Biomedical Research Institute; Hematology-Oncology Unit, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School, Boston.

STRUCTURE OF MACROPHAGE ACTIN-BINDING PROTEIN MOLECULES IN SOLUTION AND INTERACTING WITH ACTIN FILAMENTS

Evidence is presented in this paper that actin-binding protein is a dimer, which has the capacity to initiate and propagate isotropic actin filament networks. For this particular study, the structure of actin-binding molecules was examined in solution and interacting with actin filaments. At physiological ionic strength, actin-binding protein has a M_r value of 540×10^3 as determined by direct and indirect hydrodynamic measurements. It is an assymetrical dimer composed of 270×10^3 dalton subunits. Viewed in the electron microscope after negative staining or low angle shadowing, actin-binding protein molecules assume a broad range of conformations varying from closed circular structures to fully extended strands. All configurations are apparently derived from the same structure which consists of two monomer chains connected end-to-end. Other observations noted in this paper indicate that actin-binding protein dimers are extremely flexible. In further studies, direct visualization of actin-binding protein molecules between actin filaments in the electron microscope showed that dimers are sufficient for crossbridging of actin filaments and that actin-binding protein dimers are bipolar, composed of monomers connected head-to-head and having actin-

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binding sites located on the free tails. Overall, it seems apparent from these studies that actin-binding protein is a dimer at physiological ionic strength; each dimer has two actin filament binding sites, and is, therefore, sufficient to get actin filaments in solution. The length and flexibility of the actin-binding protein subunits render this molecule structurally suited for the crosslinking of large helical filaments into isotropic networks.

Hartwig, J. H. and *Stossel, T. P.*

Journal of Molecular Biology 145:563-581, 1981.

Other support: U. S. Public Health Service.

From the Department of Medicine, Massachusetts General Hospital, Boston.

DISTRIBUTION OF ACTIN-BINDING PROTEIN AND MYOSIN IN POLYMORPHONUCLEAR LEUKOCYTES DURING LOCOMOTION AND PHAGOCYTOSIS

There is a theory that actin and proteins that associate with it are the major elements of locomotion and phagocytosis by polymorphonuclear (PMN) leukocytes; and this theory is well supported by the material presented here. In this study, indirect immunoflourescence was used to examine the redistribution of myosin and actin-binding protein (ABP) molecules in rabbit PMN leukocytes during locomotion and phagocytosis. In unpolarized PMN leukocytes, ABP and myosin had a diffuse distribution with some predilection for the cortex. In polarized PMN leukocytes crawling toward yeast particles, myosin and ABP staining concentrated in the anterior pseudopod. In PMN leukocytes fixed during phagocytosis of the yeast particles, antimyosin and anti-ABP staining concentrated strikingly in the distal portions of the pseudopod embracing the yeasts. Staining for catalase, a cytoplasmic protein in PMN leukocytes, for lactoferrin, a protein of specific granules, and for myeloperoxidase, a protein of azyrophilic granules, was not concentrated in pseudopods. Taken together with available morphologic and biochemical information, these findings are consistent with a mechanism wherein interactions of actin, ABP and myosin redistribute cortical cytoplasm into pseudopods involved in locomotion and phagocytosis.

Valerius, N. H., Stendahl, O., Hartwig, J. H. and *Stossel, T. P.*

Cell 24:195-202, 1981.

From the Department of Medicine, Harvard Medical School, and the Hematology-Oncology Unit, Massachusetts General Hospital, Boston.

IDENTIFICATION OF GELSOLIN, A Ca^{2+}-DEPENDENT REGULATORY PROTEIN OF ACTIN GEL-SOL TRANSFORMATION, AND ITS INTRACELLULAR DISTRIBUTION IN A VARIETY OF CELLS AND TISSUES

Gelsolin, a 91,000-dalton globular protein from rabbit lung macrophages, has been shown before to be a major Ca^{2+}-dependent regulatory protein of actin gel-sol

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transformation. In the work reported here, antiserum prepared against gelsolin was used to detect the presence of proteins immunologically related to gelsolin in a variety of cells and tissues. Results showed that a single band of cross-reactive material which comigrated with macrophage gelsolin was found in at least nine different kinds of cells and tissues derived from rabbits and humans and in four lines of cultured cells from humans and rats. Gelsolin was also identified in human serum and plasma, raising the possibility that it may contribute to the clearance of actin from the circulatory system. Related studies, using indirect immunoflourescent staining of acetone-fixed macrophages and polymorphonuclear leukocytes, showed that gelsolin resides in the cortical cytoplasm and that during phagocytosis it is concentrated in pseudopodia engulfing particles to be ingested, an area of the cytoplasm actively engaged in movement. In longitudinal cryostat sections of contracted rabbit skeletal muscle, antigelsolin staining was associated with the I-band of the myofibril, suggesting that it may be involved, by an as yet undefined mechanism, in skeletal muscle function. In conclusion, these findings are compatible with the idea that gelsolin is an integral part of the motile apparatus of phagocytic cells and that it regulates cell movement by changing the consistency of the cytoplasm.

Yin, H. A., Albrecht, J. H. and Fattoum, A. (*Stossel, T. P.*)

The Journal of Cell Biology 91:901-906, 1981.

Other support: U. S. Public Health Service and the Edwin S. Webster Foundation.

From the Hematology-Oncology Unit, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School, Boston.

ACTIN FILAMENTS AND SECRETIONS: THE MACROPHAGE MODEL

In this fact-filled book chapter, the relationship between actin filaments and secretion is scrutinized in several different ways. In one chapter section, morphological and pharmacological evidence is presented for the participation of actin microfilaments in secretion; while, in another section, a macrophage model is employed to characterize the demonstrable association of microfilament rearrangement and secretion, it is noted here that, if the cortical actin microfilament lattice of macrophages is to have some active or passive function in secretion, it must be capable of directional movement. The elements of directional movement are (1) a force-generating mechanism, (2) an orienting influence on the force to provide directionality, and (3) a control mechanism exerted on the force-generating system or on directionality or on both. Each one of these directional movement elements is presented in fuller detail here. In summary, the force-generating mechanism is a superprecipitation of actin and myosin filaments, a process requiring hydrolysis of ATP and presumably based on the sliding-filament interaction chacterized in striated muscle. This energy-dependent mechanism may be a major consumer of the macrophage's metabolic activity and can account for the susceptibility of secretion to inhibition by metabolic poisons. Directionality and amplification of the force generated arise for controlled focal changes in the crosslinking of actin filaments. Gelsolin, a calcium-activated protein, controls lattice rigidity of

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actin by severing actin filaments between points of crosslinking by actin-binding protein. The free calcium concentrations that regulate the activity of this protein are levels found in living cells. This mechanism for directional cytoplasmic movement control by calcium is called the "Tug-of-War" hypothesis, and evidence that this mechanism exists in macrophages is presented in this paper.

Stossel, T. P.

Methods in Cell Biology 23:215-229, 1981.

Other support: U.S. Public Health Service, the Edwin S. Webster Foundation and Edwin W. Hiam.

From the Hematology-Oncology Unit, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School, Boston.

THE MOTOR OF PHAGOCYTIC LEUKOCYTES

There are three things that are needed for cytoplasmic movement: (1) force generation, (2) orientation of the force to provide direction and (3) a control mechanism. The present review summarizes information concerning the biochemical components of the motor of mammalian phagocytes and how they can interact to generate directional movement. In the first place, the motor region of phagocytic leukocytes appears to reside in the peripheral cytoplasm beneath the plasma membrane. Transmission electron micrographs of thin sections of fixed phagocytic leukocytes reveal that this cortical cytoplasm consists primarily of filamentous material, and that the filaments have the dimensions of actin polymers. These actin filaments seem to form a meshwork in which the filaments intersect or overlap at random intervals. Myosin molecules (about 1% of the total protein) of phagocytic leukocytes can form bipolar filaments at physiologic pH and ionic strength, and these myosin filaments bind to actin filaments. Although all of the evidence is not in yet, at this time it seems possible that: (1) the macrophage cortex is an isotropic lattice of actin filaments cross-linked by a protein called actin-binding protein. (2) Myosin filaments, dispersed together with magnesium and ATP throughout the lattice, exert tension on the lattice by means of the crossbridge mechanism, and (3) depending on the calcium concentration, gelsolin, a recently discovered macrophage protein, regulates the structure of the lattice. Comparisons are made in this paper between the motor of mammalian phagocytes and the motor of muscle fibers, and consideration is given to the possible importance of this motor mechanism in cancer biology.

Stossel, T. P., Hartwig, J. H. and Yin, H. L.

In: Saunders, Daniels, Serrou, Rosenfeld, and Denny (eds,): *Fundamental Mechanisms in Human Cancer Immunology*, New York: Elsevier North Holland, Inc., 1981, pp. 259-273.

Other support: U.S. Public Health Service, the Edwin S. Webster Foundation, Edwin W. Hiam, and C. L. and J. D. Kaufman.

From the Hematology-Oncology Unit, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School, Boston.

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DETERMINANTS OF FORCED EXPIRATORY FLOWS IN NEWBORN INFANTS

This study was undertaken to delineate the mechanical properties of the lung in early life by examining the determinants of forced expiratory flows and the response of flows to HeO_2 in newborn boys and girls. Specifically, maximal flows at functional residual capacity ($\dot{V}max_{FRC}$) from partial expiratory flow-volume (PEFV) curves (achieved with rapid compression of the chest) were obtained on 11 healthy newborn babies. Mean$\dot{V}max_{FRC}$, size corrected by dividing absolute values by measured thoracic gas volume (TGV), was 1.90 TGVs/s. Specific upstream conductances were high, and the cross-sectional area of the flow-limiting segment was estimated to be approximately 0.30 cm^2 in the three infants on whom recoil pressures at FRC were also measured. The cross-sectional area of the major bronchi in the neonate is approximately 0.26-0.30 cm^2. PEFV curves were convex to the volume axis. Many of the neonates increased their flows while breathing a helium-oxygen gas mixture. These results suggest (1) size-corrected flows are higher in the neonate than in older children or adults; (2) the site of the flow-limiting segment at FRC during maximal expiratory maneuvers is in large proximal airways, similar to the adult; and (3) the relationship of airway size to parenchymal size may be similar in neonates and adults or, in fact, airways may be larger, relative to parenchyma, in neonates. Therefore, these physiological data do not support the hypothesis, based on pathological studies, that peripheral airways are disproportionately smaller (when compared with central airways) in infants than in adults.

Taussig, L. M. et al.

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 53(5):1220-1227, 1982.

Other support: National Institutes of Health.

From the Department of Pediatrics, Hadassah University Hospital, Mt. Scopus, Jerusalem, Israel.

CAPTOPRIL: ASSOCIATION WITH FETAL DEATH AND PULMONARY VASCULAR CHANGES IN THE RABBIT (41446)

Captopril (D-3-mercapto-2-methylpropanoyl-L-proline) is a rather new, orally effective inhibitor of angiotensin converting enzyme, which has recently been approved for the treatment of refractory systemic hypertension. Administration of this agent has been shown experimentally to decrease circulating levels of angiotensin II and to increase levels of bradykinin and prostaglandins. For the study presented here, the effect of oral captopril on fetal survival was assessed in pregnant adult New Zealand White rabbits (20 treated with captopril and 12 controls). Results showed that fetal death in the treated rabbits was 86%, in contrast to 1% in control rabbits. Some of the rabbits were made hypoxic in a hypobaric chamber (522 mm Hg pressure) during the period of captopril administration. Under these conditions, captopril administered to the maternal rabbits had a demonstrable cardiopulmonary effect in the neonates, as shown by a significant reduction in pulmonary arteriolar medial thickness and both left and right ventricular weights compared to the hypoxic untreated controls. In view of these observations, it would be prudent to avoid using captopril for the treatment of

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hypertension during pregnancy, until the mechanism of fetal death and the reasons for species variation are known.

Keith, I.M., *Will, J. A.* and Weir, E. K.

Proceedings of the Society for Experimental Biology and Medicine 170(3):378-383, 1982.

National Institutes of Health.

Other support: From the Departments of Veterinary Science, College of Agricultural and Life Sciences and Anesthesiology, Medical School, University of Wisconsin, Madison, and the Veterans Administration Medical Center, University of Minnesota, Minneapolis.

STANDARDIZATION OF FORMALDEHYDE-INDUCED FLUORESCENCE AND ITS MEASUREMENT TO QUANTIFY SEROTONIN EMISSION IN PULMONARY NEUROENDOCRINE CELLS

The formaldehyde-induced fluorescence (FIF) method is widely used for visualization and quantitative analysis of biogenic amines in tissues. In the paper presented here, a modified and standardized quantitative FIF procedure for producing fluorophores and measuring emission intensity of serotonin-containing neuroepithelial bodies (NEBs) in the rabbit lung is described. This technique, using epifluorescence, was reproduced without significant differences between control groups. Important considerations for reproducibility were: using the same humidity (80% RH) and reaction time (2 hrs.) during the vapor treatment, sectioning at constant relative humidity, avoiding unnecessary heating (sections should not be stretched over a hot plate) and avoiding exposure of sections to light. Optimal emission readings were obtained with sectioning and mounting at 40-50% RH. Readings were reduced by 25% when the mercury light source was switched from 200 W to 100 W. It was also important to let the instruments warm up long enough to avoid drift during quantitation. Each NEB should be subjected to the same duration of light exposure for alignment (30 s) before measuring fluorescence to avoid differences from photodecomposition.

Keith, I. M., Wiley, L. A. and *Will, J. A.*

Histochemistry 75:253-258, 1982.

Other support: College of Agriculture and Life Sciences, University of Wisconsin, Madison.

From the Department of Veterinary Science, University of Wisconsin, Madison.

PLASMA PANCREATIC TRYPSINOGENS IN CHRONIC RENAL FAILURE AND AFTER NEPHRECTOMY

The study presented here had three major objectives: (a) to investigate the relation between plasma pancreatic anionic and cationic trypsinogen in patients with chronic renal failure or nephrectomy, (b) to determine whether hemodialysis alters the plasma levels of these zymogens in patients with chronic renal failure (CRF) or nephrectomy, and (c) to study the effects of nephrectomy on the plasma trypsinogen response to hormonal stimulation of pancreatic zymogen secretion in an established animal model,

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the dog. For this study, plasma concentrations of anionic and cationic trypsinogen were measured in CRF and anephric patients. Results showed that the plasma concentrations were significantly elevated in both groups of patients. Hemodialysis did not change their plasma levels. The plasma levels of anionic and cationic trypsinogens were highly correlated in patients and normal subjects; however, the relative concentrations of anionic trypsinogen were significantly higher in renal failure patients. This suggests that in patients with renal failure the secondary clearance mechanisms for these plasma proteins clear cationic molecules more efficiently than they clear anionic molecules. In a related experiment using normal dogs, intravenous infusion of synthetic octapeptide of cholecystokinin (CCK-8) resulted in small transitory increases in plasma trypsinogen levels. After nephrectomy, basal levels of anionic and cationin trypsinogen were elevated, and intravenous infusion of CCK-8 resulted in prolonged, high levels of plasma trypsinogens.

Geokas, M. C. et al.

American Journal of Physiology 242 (Gastrointest. Liver Physiol. 5):G177-G182, 1982.

Other support: Veterans Administration and the National Institutes of Health.

From Martinez Veterans Administration Medical Center, Martinez, CA; Departments of Internal Medicine and Biological Chemistry, University of California Davis School of Medicine, Davis; and the Veterans Administration Wadsworth Medical Center, and Department of Surgery, UCLA School of Medicine, Los Angeles.

III. *Heart and Circulation*

SEROTONIN RECEPTOR-MEDIATED STIMULATION OF BOVINE SMOOTH MUSCLE CELL PROSTACYCLIN SYNTHESIS AND ITS MODULATION BY PLATELET-DERIVED GROWTH FACTOR

This paper reports that serotonin, an indoleamine released from platelet dense bodies, stimulates prostacyclin (PGI_2) production by vascular smooth muscle cells in culture through a specific serotonin-receptor-mediated mechanism. It further reports that serotonin and platelet-derived growth factor (PDGF) act synergistically to elicit dramatic increases in PGI_2 synthesis by vascular smooth muscle cells. In the basic work that led to these conclusions, serotonin (5-hydroxytryptamine: 0.5 μM and above) stimulated the synthesis of PGI_2 (as measured by radioimmunoassay of 6-ketoprostaglandin $F_1\alpha$) by bovine aortic smooth muscle cells in culture. This effect was structurally specific; a similar response was not elicited by the other indoles or by the amines phenylephrine, isoproterenol, dopamine, or histamine. The response was reversible

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and was saturable at serotonin concentrations of 10 μM or higher. An increase in PGI_2 synthesis was elicited by the addition of a serotonin agonist, quipazine, and antagonized by the serotonin receptor blockers cyproheptadine, methysergide, or methiothepin. The addition of PDGF to cultures of smooth muscle cells dramatically enhanced PGI_2 synthesis in response to the coadministration of serotonin. PDGF greatly increased the maximum response to serotonin without altering the half-maximal effective concentration for serotonin. Also, this synergistic interaction was blocked by the addition of a serotonin-receptor blocking agent.

Coughlin, S. R., Moskowitz, M. A., *Antoniades, H. N.* and Levine, L.

Proceedings of the National Academy of Sciences of the United States of America 78(11):7134-7138, 1981.

Other support: National Institutes of Health.

From the Departments of Neurosurgery and Neurology, Massachusetts General Hospital, Harvard Medical School, Boston; Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge; Center for Blood Research, Department of Nutrition, Harvard School of Public Health, Boston, and Department of Biochemistry, Brandeis University, Waltham, MA.

HUMAN PLATELET-DERIVED GROWTH FACTOR STIMULATES AMINO ACID TRANSPORT AND PROTEIN SYNTHESIS BY HUMAN DIPLOID FIBROBLASTS IN PLASMA-FREE MEDIA

There are two major points about the action of platelet-derived growth factor (PDGF) on human fibroblasts that emerge from this paper. First, stimulation of amino acid transport and [^{3}H]leucine incorporation are early effects of PDGF action on quiescent human fibroblasts. Second, the actions of PDGF on amino acid uptake and [^{3}H]leucine incorporation are brought about by PDGF alone and do not require simultaneous or subsequent presence of plasma components or other hormones. Specifically, results of this study show that purified human PDGF induces an increase in amino acid uptake via system A in quiescent human diploid fibroblasts. Cells must be exposed to PDGF for 45 min to obtain maximum transport stimulation. Transport stimulation requires protein synthesis; transient exposure to PDGF, alone, in the absence of plasma components can stimulate transport. Acid-insoluble [^{3}H]leucine incorporation is also stimulated by PDGF treatment, and this event also does not require the presence of plasma components. Finally, antiserum to PDGF that blocks PDGF-stimulated DNA synthesis in these cells also blocks PDGF-stimulated amino acid uptake and protein synthesis. Increased amino acid uptake and protein synthesis that occur soon after addition of fresh serum to quiescent cells can be attributed to the action of PDGF acting alone and should be useful as markers for the investigation of early cellular events caused by PDGF.

Owen, A. J. III, Geyer, R. P. and *Antoniades, H. N.*

Proceedings of the National Academy of Sciences of the United States of America 79(10):3203-3207, 1982.

Other support: U. S. Public Health Service and the National Cancer Institute.

From the Department of Nutrition, Harvard School of Public Health, Boston.

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A NEW HORMONAL POLYPEPTIDE THAT STIMULATES THE GROWTH OF CELLS IN CULTURE: ISOLATION AND CHARACTERIZATION FROM HUMAN SERUM AND HUMAN PLATELETS

This presentation affords a wide view of the body of Antoniades and his associates. It describes briefly (1) their early studies which led them to the discovery and isolation of a new hormonal polypeptide growth factor from human serum, which appears to be indispensable for the growth of normal cells in culture; (2) the subsequent isolation and purification of this polypeptide from clinically outdated human platelets; (3) the growth effects, and diverse metabolic activities of this purified polypeptide in cells in culture, and (4) studies in progress aimed at the elucidation of its mode of action in the growth of normal cells in culture. In the work described, studies with cell culture and a specific radioimmunoassay demonstrated that the serum polypeptide growth factor derives from platelets and is released into serum during blood clotting; it is not present in platelet-poor plasma. This human platelet-derived polypeptide growth factor (PDGF) has been purified to homogeneity from clinically outdated human platelets. The specific activity of the purified PDGF is 20 million times greater than that found in unfractionated human serum. It stimulates DNA replication and cell proliferation at a concentration of 1 ng/ml. Characterization studies have shown that PDGF is a cationic, heat-stable (100°C) polypeptide, with a pI of about 9.8. The unreduced molecule has an apparent molecular weight of about 35,000 daltons as judged by NaDodSO$_4$/polyacrylamide gel electrophoresis, and reduction results in the production of two inactive polypeptide chains of about 14,000 and 17,000 daltons respectively.

Antoniades, H. N.

In: Bing, D. H. and Rosenbaum, R. A. (eds.): *Plasma and Cellular Modulatory Proteins*, Boston: Center for Blood Research, 1980, pp. 1-14.

Other support: National Cancer Institute.

From the Center for Blood Research and Harvard University School of Public Health, Boston.

HUMAN PLATELET-DERIVED GROWTH FACTOR (PDGF): PURIFICATION OF PDGF-I and PDGF-II AND SEPARATION OF THEIR REDUCED SUBUNITS

In this methodological paper, a procedure that allows the direct recovery of biologically active human platelet-derived growth factor (PDGF) from stained gels after separation on NaDodSO$_4$/polyacrylamide gel electrophoresis is described. This technique enabled the identification and purification to homogeneity of two active PDGF polypeptides, one with a molecular weight of about 35,000 (PDGF-I) and the other with a molecular weight of about 32,000 (PDGF-II). Reduced PDGF-I produced two inactive subunits, with molecular weights of about 15,000 and 18,000. Reduced PDGF-II also produced two inactive subunits, with molecular weights of about 15,000 and 16,000. It is possible that PDGF-II derives from proteolytic cleavage of PDGF-I.

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Antoniades, H.N.

Proceedings of the National Academy of Sciences of the United States of America 78(12): 7314-7317, 1981.

Other support: National Institutes of Health.

From the Center for Blood Research and Department of Nutrition, Harvard School of Public Health, Boston.

STIMULATION OF PHOSPHOLIPID AND CHOLESTEROL ESTER SYNTHESIS BY PLATELET-DERIVED GROWTH FACTOR IN NORMAL AND HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA HUMAN SKIN FIBROBLASTS

The aim of this investigation was to study the effect of human platelet-derived growth factor (PDGF) on lipid metabolism, particularly cholesterol ester and phospholipid synthesis in normal and familial hypercholesterolemic-derived human skin fibroblasts. Results of this study show that pure PDGF at nanogram levels stimulates cholesterol ester, phospholipid and DNA synthesis in both normal and familial hypercholesterolemia mutant human skin fibroblasts. While stimulation of DNA synthesis did not begin until 15-24 hrs. after addition of PDGF to quiescent normal and FH mutant fibroblasts, stimulation of [^{3}H]oleic acid incorporation into cholesterol ester and phospholipid was evident 3-6 hrs. after the addition of PDGF. In the normal cells, the rate of cholesterol ester synthesis was maximal at 24 hrs., then rapidly declined. Cholesterol esterification was much lower in the FH cells than in the normal cells. The stimulation of [^{3}H]oleic acid incorporation into cholesterol ester by PDGF was inhibited in both normal and FH mutant skin fibroblasts by progesterone, an inhibitor of acyl-CoA: cholesterol acyltransferase. The rate of cholesterol ester synthesis in the normal cells increased as the concentration of platelet-poor plasma or low density lipoprotein (LDL) was increased, especially in the presence of PDGF. Linearization of the LDL dose-response curve indicated that PDGF increased the rate rather than the affinity of the overall cholesterol esterification system. The rate of cholesterol esterification in the FH mutant cells was highest in the absence of LDL or at low levels of platelet-poor plasma. Consequently, PDGF can stimulate cholesterol ester synthesis by LDL and non-LDL-mediated processes.

Leslie, C. C., *Antoniades, H. N.* and Geyer, R. P.

Biochimica et Biophysica Acta 711:290-304, 1982.

From the Department of Nutrition, Harvard School of Public Health, Boston.

MIGRATION OF CULTURED VASCULAR CELLS IN RESPONSE TO PLASMA AND PLATELET-DERIVED FACTORS

In the study presented here, a quantitative assay for cell migration was used to measure the response of pericytes, smooth muscle cells and endothelial cells from large and small vessels to human serum, plasma and purified platelet-derived factors.

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In the first part of this study, phagokinetic migration of cultured vascular cells was tested in response to human platelet-rich serum ('serum') and human platelet-poor plasma serum ('plasma'). The cell types tested included bovine aortic endothelial cells, human umbilical vein endothelial cells, human hemangiomal capillary endothelial cells, bovine adrenal microvascular pericytes, and bovine aortic smooth muscle cells. Human serum stimulated a significant increase in the rate of migration in all five cell types. Human plasma stimulated the endothelial cells to migrate but had no effect on the migration of pericytes or smooth muscle cells. In the second part of this study, highly purified platelet-derived growth factor (PDGF) stimulated dose-dependent migration of smooth muscle cells causing a 50% increase in phagokinetic track area relative to controls. Neither pericyte nor endothelial cell migration was stimulated by PDGF. Rabbit antiserum to human PDGF completely blocked the smooth muscle cell migration induced by either 10% serum of lng/ml pure PDGF. Purified platelet factor IV (PF_4) stimulated migration of pericytes but not of smooth muscle cells or endothelial cells. Sheep antiserum to human PF_4 completely blocked the pericyte migration induced by either 10% serum or 1 ug/ml pure PF_4. These results indicate that PDGF is the primary factor in serum responsible for the migration of cultured aortic smooth muscle cells and that PF_4 is a critical factor required to induce the migration of pericytes. Other factors present in both plasma and serum control the migration of vascular endothelial cells.

Bernstein, L. R., *Antoniades, H.* and Zetter, B. R.

Journal of Cell Science 56:71-82, 1982.

Other support: National Institutes of Health.

From the Departments of Physiology and Surgery, Harvard Medical School, Children's Medical Center, and Harvard School of Public Health, Center for Blood Research, Boston.

PLATELET-DERIVED GROWTH FACTOR BINDS SPECIFICALLY TO RECEPTORS ON VASCULAR SMOOTH MUSCLE CELLS AND THE BINDING BECOMES NONDISSOCIABLE

Platelet-derived growth factor (PDGF) is a potent stimulant of the growth of vascular smooth muscle. In order to understand the action of PDGF, methods had to be developed for identifying PDGF receptor sites. To accomplish this, radioiodinated PDGF (^{125}I-PDGF) was used in studies of PDGF binding sites on vascular smooth muscle cells. There was an excellent correlation between the ability of ^{125}I-PDGF to stimulate cell proliferation and to bind specifically to cultured vascular smooth muscle cells. The half-maximal concentration for both processes was 0.1 nM. There were 50,000 binding sites per cell. Reduced PDGF, prepared by treatment of PDGF with 20 mM dithiothreitol, had neither the ability to bind to smooth muscle cells nor to stimulate cellular proliferation. Epidermal growth factor, nerve growth factor, fibroblast growth factor, and histone B did not compete for the binding sites at a concentration of 10 nM. ^{125}I-PDGF binding was slowly reversible at 4°C and was rapidly and totally

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reversible after a 1-min incubation at 37°C. After continued incubation at 37°C, the binding became irreversible. The half-time for formation of the nondissociable state of ^{125}I-PDGF was ≈ 5 min at 37°C. The nondissociable state of binding was not formed at 4°C even after 1 hr of incubation. These data suggest that the labeled sites are the PDGF receptors that mediate PDGF's mitogenic action and that a nondissociable state of PDGF binding is formed at 37°C. It is likely that nondissociable PDGF represents internalized ligand or binding to sites that are converted to a high-affinity state after the ligand binds.

Williams, L. T., Tremble, P. and *Antoniades, H. N.*

Proceedings of the National Academy of Sciences of the United States of America 79:5867-5870, 1982.

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CONTRIBUTION OF PLASMA PROTEASE INHIBITORS TO THE INACTIVATION OF KALLIKREIN IN PLASMA

This study was designed to assess the respective contribution of the different plasma protease inhibitors to the inactivation of kallikrein in plasma. The use of a new technique that selectively inactivates α_1M in plasma facilitated the comparison of the kinetics of kallikrein inactivation. Results showed that, under pseudo-first-order conditions, the inactivation rate constant of kallikrein in normal plasma was 0.60 min^{-1}. This rate constant was reduced to 0.35, 0.30, and 0.06 min^{-1}, in plasma deficient respectively in C$\bar{1}$-INH, α_2M, or both inhibitors. Thus C$\bar{1}$-INH (42%) and α_2M (50%) were found to be the major inhibitors of kallikrein in normal plasma. Moreover, all the other protease inhibitors present in normal plasma contributed only for 8% to the inactivation of the enzyme. To confirm these kinetic results, ^{125}I-Kallikrein (M, 85,000) was completely inactivated by various plasma samples, and the resulting mixtures were analyzed by gel filtration on Sepharose 6B CL for the appearance of ^{125}I-kallikrein-inhibitor complexes. Overall, the results presented here indicate that C$\bar{1}$-INH and α_2M are the major inhibitors of kallikrein in normal human plasma. This conclusion is supported by (a) the analysis of the kinetics of kallikrein inactivation in normal and protease inhibitor-deficient plasma, and (b) by the quantitation upon gel filtration of the kallikrein-inhibitor complexes formed in plasma as the result of the inactivation of purified radiolabeled enzyme.

Schapira, M., Scott, C. F. and *Colman, R. W.*

Journal of Clinical Investigation 69:462-468, 1982.

Other support: National Institutes of Health.

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HIGH MOLECULAR WEIGHT KININOGEN OR ITS LIGHT CHAIN PROTECTS HUMAN PLASMA KALLIKREIN FROM INACTIVATION BY PLASMA PROTEASE INHIBITORS

Five plasma protease inhibitors are known to inactivate kallikrein, but now it has been shown also that high molecular weight kininogen (HMWK) or its light chain can act to protect kallikrein from inactivation by these inhibitors. For this biochemical study, the kinetics of the inactivation of kallikrein by α_2-macroglobulin, antithrombin III, and α_1-antitrypsin were noted in the presence and absence of HMWK. In the absence of HMWK, the second-order rate constants k_{+2}/K_i for the inactivation were respectively 6.9×10^3, 1.8×10^4, and $2.5 \times 10^2 M^{-1}min^{-1}$. When HMWK was present during the reaction, the inactivation rates by these plasma protease inhibitors were reduced as a result of the formation of kallikrein-high molecular weight kininogen complex, $K_d = 0.75$ μM. When the light chain derived from reduction of kinin-free HMWK was substituted for the parent molecule, a more pronounced reduction in inactivation rates was observed due to the formation of a kallikrein-light chain complex ($K_d = 0.14$ μM). These results demonstrate that the combining site for kallikrein on HMWK, responsible for complex formation and protection against inhibitors, resides in the light-chain region of the molecule. Moreover, kallikrein appears to bind more tightly to the light chain of HMWK than to the parent molecule.

Schapira, M., Scott, C. F., James, A., Silver, L. D., Kueppers, F., James, H. L., and *Colman, R. W.*

Biochemistry 21(3):567-572, 1982.

Other support: National Institutes of Health.

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REGIONAL RENAL AND SPLANCHNIC BLOOD FLOWS DURING NICOTINE INFUSION: EFFECTS OF *ALPHA* AND OF COMBINED *ALPHA* AND *BETA* ADRENERGIC BLOCKADE

This study was instituted for the purpose of defining directly the roles of *alpha* adrenergic and possible nonadrenergic mechanisms in vasoconstrictor responses to nicotine. To do this, nicotine-induced changes in blood flow and vascular conductance in regional renal and splanchnic beds were evaluated before and after (1) selective blockade of *alpha* adrenergic receptors and (2) combined blockade of *alpha* and *beta* adrenergic receptors. Before adrenergic blockade, nicotine increased arterial pressure (+82%) but had heterogeneous directional effects on regional blood flows: pancreas (−64%), duodenum (−33%), kidney cortex (−31%), kidney medulla (−17%), liver (+5%) and spleen (+71%). Vascular conductance was reduced in kidney cortex, kidney medulla, duodenum, liver and pancreas, and was not altered in spleen. Selective *alpha* adrenergic blockade prevented the hypertensive response to nicotine, but heterogeneous changes in regional flows persisted. After combined *alpha* and *beta* adrenergic blockade, nicotine increased systemic arterial pressure and decreased

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